

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:
expressing SP2/0 cells that are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or V κ domain in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, where the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose, so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site.

2. (Canceled)

3. (Canceled)

4. (Original) The method of claim 1, wherein the antibody has more than one glycosylation site.

5. (Original) The method of claim 1, wherein the antibody is a single-chain antibody.

6. (Currently amended) A method of making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site comprising:
expressing SP2/0 cells that are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or V κ domain in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, wherein said ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose, so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody to produce a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

7. (Original) The method of claim 6, wherein the fragment is an F(ab')₂ fragment.

8. (Previously presented) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

reacting a glycosylated antibody produced according to claim 1 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

9. (Original) The method of claim 8, wherein the antibody is purified before reaction with the agent.

10. (Original) The method of claim 8, wherein the agent is added directly to the culture medium.

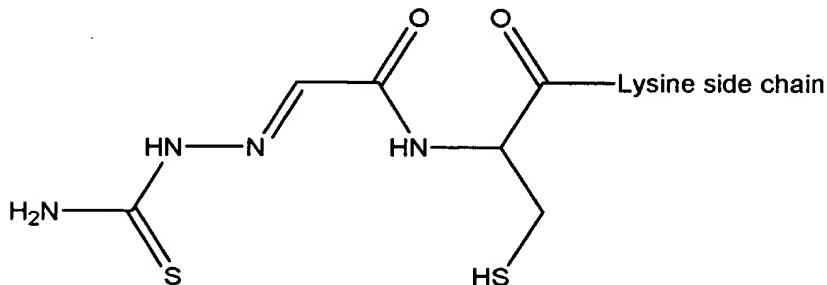
11. (Original) The method of claim 8, wherein the antibody is immobilized on a protein A column prior to reaction with the agent, and eluted from the protein A column after reaction with the agent.

12. (Original) The method of claim 8, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

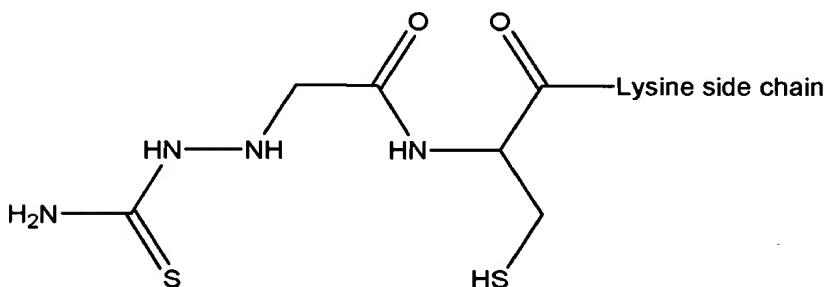
13. (Original) The method of claim 12, wherein the agent is selected from the group consisting of peptides, oligosaccharides, biotinamidocaproyl hydrazides, diagnostic markers, drugs, toxins, imaging radioisotopes, and therapeutic radioisotopes.

14. (Original) The method of claim 8, wherein the agent is a ligand-containing peptide selected from the group consisting of diethylene triamine pentaacetic acid-bearing

(DTPA-bearing) peptides, 1,4,7,10-tetraazacyclododecane-N,N',N''N'''-tetraacetic acid-bearing (DOTA-bearing) peptides, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TscGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{CONH-NH}_2$, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TsdGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{H}(\text{NH}_2)\text{CONH-NH}_2$, and $\text{H}_2\text{N-NH-CH}_2\text{-CO-D}_d\text{-K}_d(\text{TscGC})\text{-D}_d\text{-K}_d\text{-NH}_2$, where K_d and D_d represent the D-amino acids D-lysine and D-aspartic acid, respectively, and where TscGC is the ligand:



and TsdGC is the ligand:



15. (Withdrawn) The method of claim 14, wherein the agent is $\text{H}_2\text{N-NH-CH}_2\text{-CO-D}_d\text{-K}_d(\text{TscGC})\text{-D}_d\text{-K}_d\text{-NH}_2$.

16. (Previously presented) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

reacting a glycosylated antibody fragment produced according to claim 6 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

17. (Original) The method of claim 16, wherein the fragment is an F(ab')₂ fragment.

18. (Original) The method of claim 16, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

19. (Previously presented) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, wherein said glycosylated site is in the V κ or CH1 domain, and wherein the reactive ketone group is not introduced by oxidation.

20. (Canceled)

21. (Original) The glycosylated antibody or antigen-binding antibody fragment of claim 19, wherein the antibody or antibody fragment has more than one glycosylation site, each of which has a reactive ketone group.

22. (Previously presented) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through the glycosylated site, wherein said glycosylated site is in the V κ or CH1 domain, and wherein the agent is conjugated to a reactive ketone group on the glycosylated site that is not introduced by oxidation.

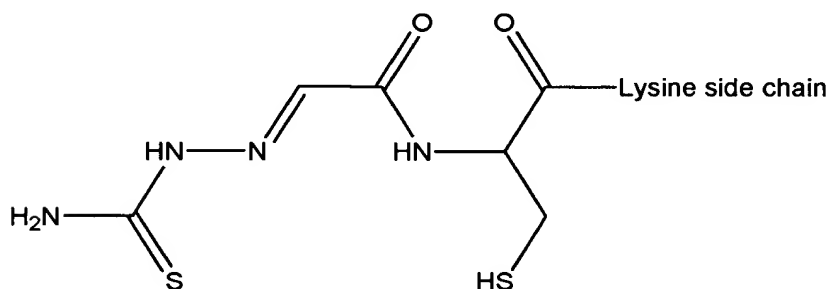
23. (Original) The immunoconjugate of claim 22, wherein the glycosylated site is selected from the group consisting of HCN1, HCN5 and V κ -N.

24. (Original) The immunoconjugate of claim 22, wherein the antibody has more than one glycosylated site, each of which is conjugated to an agent.

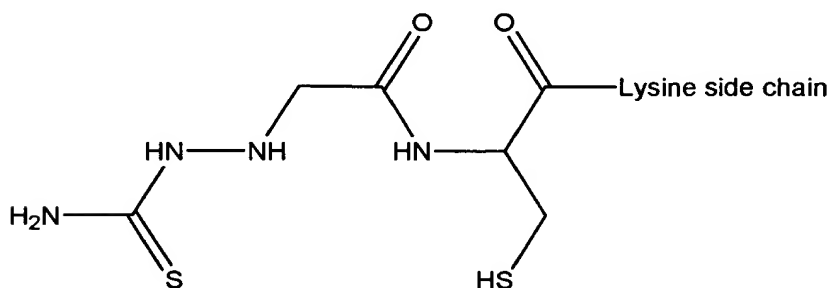
25. (Original) The immunoconjugate of claim 22, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

26. (Original) The immunoconjugate of claim 25, wherein the agent is selected from the group consisting of peptides, oligosaccharides, biotinamidocaproyl hydrazides, diagnostic markers, drugs, toxins, imaging radioisotopes, and therapeutic radioisotopes.

27. (Original) The immunoconjugate of claim 25, wherein the agent is a ligand-containing peptide selected from the group consisting of DTPA-bearing peptides, DOTA-bearing peptides, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TscGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{CONH-NH}_2$ and $\text{AcK}_d\text{D}_d\text{K}_d(\text{TsdGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{H}(\text{NH}_2)\text{CONH-NH}_2$, where K_d and D_d represent the D-amino acids D-lysine and D-aspartic acid, respectively, and where TscGC is the ligand:



and TsdGC is the ligand:



28. (Withdrawn) The immunoconjugate of claim 27, wherein the agent is $\text{H}_2\text{N-NH-CH}_2\text{-CO-D}_d\text{-K}_d\text{-(TscGC)-D}_d\text{-K}_d\text{-NH}_2$.

29. (Original) The immunoconjugate of claim 22, wherein the agent is a chelating agent chelated to a diagnostic or therapeutic radioisotope.

30. (Withdrawn) A method of targeting an active agent to an *in vivo* target site comprising administering an immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an active agent through a reactive ketone group on a glycosylated HCN1, HCN5 or V κ -N glycosylation site and not as a conjugate to an oxidized sugar.

31. (Withdrawn) The method of claim 30, wherein the active agent is selected from the group consisting of diagnostic and therapeutic agents.

32. (Withdrawn) The method of claim 30, wherein the antibody or antibody fragment has multiple glycosylated sites, each of which is conjugated to an active agent.

33. (Withdrawn) A method of targeting an active agent to an *in vivo* target site comprising:

administering a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on a HCN1, HCN5 or V κ -N glycosylation site, and allowing the antibody or antibody fragment to localize at the target site;

optionally, administering a clearing agent to clear non-localized antibody or antibody fragment from circulation; and

administering an active agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, whereby the active agent localizes at the target site via conjugation with the pre-targeted antibody or antibody fragment.

34. (Withdrawn) The method of claim 33, wherein the active agent is selected from the group consisting of diagnostic and therapeutic agents.

35. (Withdrawn) The method of claim 33, wherein the clearing agent is administered.

36. (Withdrawn) The method of claim 35, wherein the clearing agent is an anti-idiotypic clearing agent.

37. (Withdrawn) The method of claim 33, wherein the antibody or antibody fragment has more than one glycosylated site, and wherein more than one active agent moiety is conjugated to the pretargeted antibody or antibody fragment.

38. (Currently amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

expressing SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or V κ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, where the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose, so that they produce an N-glycosylated antibody having a reactive ketone group on the glycosylated site.

39. (Canceled)

40. (Canceled)

41. (Currently amended) A method making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, comprising:

expressing SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or V κ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, where the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose, so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody into a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

42. (Canceled)

43. (Canceled)

44. (Previously presented) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

reacting a glycosylated antibody according to claim 38 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

45. (Canceled)

46. (Previously presented) A method according to claim 44, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

47. (Previously presented) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

reacting a glycosylated antibody fragment according to claim 41 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

48. (Canceled)

49. (Previously presented) A method according to claim 47, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

50. (Canceled)

51. (Canceled)

52. (Canceled)

53. (Previously presented) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and V κ -N, and wherein the reactive ketone group is not introduced by oxidation.

54. (Previously presented) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through a reactive ketone on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and V κ -N, and wherein the reactive ketone group is not introduced by oxidation.

55. (Previously presented) A glycosylated antibody having a reactive ketone group on a glycosylated site, prepared by a method as recited in claim 1, wherein the reactive ketone group is not introduced by oxidation.